Report of the Haffkine Institute

for the Year 1927

by

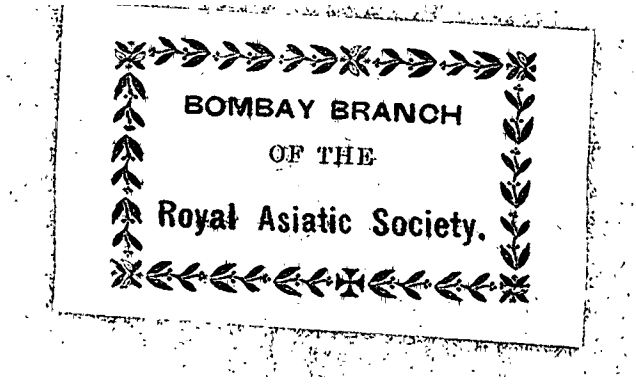
LIEUT.-COLONEL F. P. MACKIE, I.M.S.,
Director, Haffkine Institute

[Price—Annas 14 *or* 1*s.* 6*d.*]

LIBRARY
OF THE
BOMBAY
BRANCH
OF THE
ROYAL ASIATIC SOCIETY

BOMBAY
PRINTED AT THE GOVERNMENT CENTRAL PRESS
1928.

Obtainable from the Superintendent of Government Printing and Stationery, Bombay; from the High Commissioner for India, 42 Grosvenor Gardens, S.W.1, London; or through any recognized Bookseller



Digitized with financial assistance from the Government of Maharashtra on 11 July, 2017

CONTENTS

	Page
Staff	1
General Review	2
Haffkine's Plague Prophylactic Vaccine	7
Pharmacological Unit	17
Biochemical Unit	19
Chemical Department	22
Antirabic Department	24
Vaccine Department	25
General Diagnostic Department	27
Pathological Section	28
Special Researches financed by the Indian Research Fund Association ...	29
Researches on Sprue	30
Anti-plague Inquiry	31
Inquiry on maternal morbidity and mortality in childbirth	38
Schistosomiasis	40
Appendix	42

LIBRARY OF THE BOMBAY BRANCH OF THE ROYAL ASIATIC SOCIETY.

CORRECTION SLIP

On page 1 under *Research Officers* below Dr. Agnes Murphy *add*—

Dr. M. Mehta, D.Sc. (Lond.).

REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1927

STAFF

Director

Lt.-Col. F. P. Mackie, V.H.S., O.B.E., M.D., M.Sc., D.P.H F.R.C.S., F.R.C.P., I.M.S.

Assistant Directors

Major S. S. Sokhey, M.A., B.Sc., M.D. (Edin.), D.T.M. & H. (Eng.) I.M.S., in charge Biochemical Unit.
Dr. S. N. Gore, L.M. & S.

Pharmacological Unit

Rev. Father J. F. Caius, S.J., F.L.S., M.S.C.I. (Paris).
Dr. K. S. Mhaskar, M.A., B.Sc., M.D., D.P.H., D.T.M. & H.

Biochemical Unit

Mr. M. A. Malandkar, M.A., M.Sc.
Mr. S. K. Gokhale, B.A., M.Sc., A.I.I.Sc.

Antirabic Department

Military Assistant Surgeon H. M. La Frenais, I.M.D.
Mr. R. K. Gokhale, S.M.S.

Research Officers

Dr. Margaret Balfour, C.B.E., M.D.
Dr. Agnes Murphy, W.M.S., from 1st January to 14th March 1927.
Mrs. Emanuelov, from 15th March to 31st December 1927.
Dr. B. P. B. Naidu, M.D. (Edin.), M.Hy., D.P.H., D.T.M. (L'pool).
Dr. S. A. Kamat, Ph.D. (Berlin), from 1st January to 8th May 1927.
Dr. J. H. Wadia, B.A. (Hons.), B.Sc. (Bom.), Ph.D. (Cantab.), from 9th March to 31st December 1927.
Dr. H. S. Billimoria, M.B., B.S., D.T.M. (L'pool), from 1st February to 31st December 1927.

Medical and Scientific Assistants

Khan Bahadur C. R. Avari, B.M.S. (General Diagnostic Department).
Mr. G. D. Chitre, L.M. & S., B.M.S. (Sprue and Plague Research).
Dr. N. P. Dalal, M.B., B.S., D.P.H. (Lond.), D.T.M. & H. (Eng.), from 16th November 1927 to 31st December 1927 (on leave from 1st September 1926 to 15th November 1927).
Jamadar Shamsher Jung, I.M.D. (Plague Research).
Mr. T. G. Akula, S.M.S. (Morbid Histology and Malaria).
Jamadar F. Jasudasan, I.M.D. (Bilharziasis).
Mr. K. H. Bharucha, B.A., B.Sc. (Public Health Chemistry).
Mr. K. J. Tanna, S.M.S., acting from 1st January to 31st August 1927.
Mr. R. N. Oza, S.M.S., acting from 14th November to 15th December 1927.

Chief Technical Assistants

Mr. A. Petty (Plague Vaccine).
„ J. Petty (do.).
„ J. Mallett (do.).
„ F. R. Mistri—Head Clerk.
„ G. G. Subnis—Accountant.
„ R. D. Panwalkar, A.M.—Artist and Photographer.
„ S. V. Nadkarni—Storekeeper.
„ S. R. Rajadhyax—Packing.
„ H. J. Parelkar—Engineer.
„ Shaik Mahomedalli—Senior Media Maker.
Junior staff, subordinates and menials numbering 120.

General Review

Plague.—The demand for vaccine has dropped heavily during the current year, being little more than one-third of last year's output. Though this is a loss to the financial side of the Institute, in every other way it is a matter for congratulation as indicating a lower incidence of the disease throughout India. A full report of the Institute's activities in Plague during the last few years has been collected by Dr. Naidu and much information, in an easily digestible form, will be found later in this report.

The variation of potency in different brews of Haffkine's vaccine has been the subject of close study and it will be shown that 32 per cent. of rats are saved from death by the vaccine compared with unprotected rats of which only 6·3 per cent. survive the minimal lethal dose of plague bacilli. By attention to the quality of the strains used in making the vaccine the potency of the different brews has been increased and none have had to be discarded on this account. The antigenic quality of different strains or races of the plague bacillus varies considerably and by eliminating "rough" or "giant" colonies an increase in the prophylactic power is being effected.

At the instance of the Director of Public Health, Punjab, some experiments were made to ascertain whether vaccine stored in the Punjab underwent deterioration as compared with that stored in Bombay. It is important to note that the hot weather conditions of Northern India had no adverse effect on the quality of the vaccine so that we may affirm that vaccine may, within reasonable time limits, be stored in any part of India without deterioration.

The work of examining rats caught in the city of Bombay continues and over half a million were received during the year. It appears from the comparative table that infected rats and human cases of plague are both undergoing a progressive reduction in the city. One of the principal factors in this satisfactory state of affairs is the steadily increasing immunity to plague of the Bombay rat population. It is known that rats are immune to plague in direct proportion to the prevalence of the disease amongst themselves and

the human population. To take an instance, the rats of Bombay (which has been subjected to repeated invasion of human and rodent plague for nearly 30 years) are relatively immune to the disease, whereas in Madras, a city almost entirely free from plague, the rats are still highly susceptible.

As a result of experiments on rat immunity it was noted that in 1910, Bombay rats showed a death rate (after inoculation with plague) of 54 per cent; in 1911, 33 per cent; in 1912, 24 per cent; in 1926 and 1927, 17·6 per cent. and 18·1 per cent. In other words Bombay rats are yearly getting more and more immune and this may well be an explanation of the gradual disappearance of the disease. It has been further shown that rats which have survived an attack of plague are completely immune to large doses of bacilli as long as 180 days after their first infection. This means that during a period of rat epizootic most of the susceptible rats are killed off, those that survive are immune to further attacks, and the general level of immunity amongst the total population undergoes a progressive increase.

Anti-plague serum.—The treatment of human plague is still in a very unsatisfactory condition despite the many years of research in various parts of the world which have been directed towards producing a potent anti-plague serum. Dr. Naidu and his assistants have set themselves to study this problem anew and have succeeded in producing a serum which saves the lives of 80 per cent. of plague infected rabbits. It remains to translate these results into the human disease and researches are now being carried on into the possibility of producing a potent sheep serum in sufficient quantities to test its effect on a series of human cases.

The treatment of plague is being approached from two other directions, one by chemotherapy and the other by bacteriophage. Experiments have already been carried out by the plague department in association with the pharmacological department, in an endeavour to find a chemical substance which shall have a lethal effect on *B. pestis* in the animal body. A number of compounds have been tested but though several have been found which will kill the bacilli in the test tube in high dilutions they are found to be ineffective when introduced into the blood stream of infected animals.

Stimulated by the visits of Professor d'Herelle, the great authority on the bacteriophage principle, a number of attempts have been made to apply this treatment to human cases. So far the results are disappointing, but with improvements in the technique it is probable that better success will be obtained. Dr. Avari has particularly identified himself with this line of research.

The Pharmacological Department.—Both Father Caius and Dr. Mhaskar have been on leave during the year so that for six months the department has been closed. Some interesting and important work has been done on the chemotherapy of plague, but the results are not sufficiently advanced to justify the treatment of human beings with this form of medication. Just as Ehrlich over a period of years studied 605 compounds before he discovered his

famous 606, so this study on similar lines may only achieve success after prolonged and laborious research.

The enquiry into the value of indigenous drugs continues in the hope, faint though it be, that some such drug has so far eluded the observation of medical men and pharmacists and still remains to be run to earth. So far these researches have not revealed any Ayurvedic or Unani formula which is superior to those already in world wide use.

One of the great drawbacks to the progress of this department has hitherto been the difficulty in getting suitable cases in hospitals on which to test the efficacy of drugs. This has now been solved by the association of Dr. Yodh of the J. J. Hospital as clinical observer in the work of the pharmacological unit. This valuable addition completes the team of observers : a chemist to work out and purify the active principles of the drugs, a pharmacologist to test their effects on the vital organs of animals, and a physician to observe their effects on human patients.

The Biochemical Department.—Researches have been carried on during the year by Major Sokhey and his assistants into the basal metabolic rate in Indians and Europeans, into the hepatic and pancreatic functions in sprue, into the biochemistry of anæmia, and of certain forms of kidney disease. The work of this department is in close association with the more advanced physicians in Bombay who are aware of the rapidly growing importance of this young science to the diagnosis and treatment of disease.

This department, which owes its inception and most of its upkeep to the generosity of the Indian Research Fund Association, has now been accepted and taken over by the Bombay Government. As the first unit of medical biochemistry in India it is already fully justifying the long and strenuous parturition which preceded its birth.

The Anti-rabic Department.—There is a progressive increase in the output from this department; but, in accordance with directions received from the Government of India, no new treatment centres have been opened during the year. As a result of the recommendations of the Anti-rabic Committee of the League of Nations, held recently in Paris, experiments are being carried out on a new method of anti-rabic vaccine production; and, when the results are known, the Government of India intend calling a conference of anti-rabic experts in India to discuss the future policy, particularly as regards the broadcasting of the vaccine. Until this conference has reported its views, the extension of the present system is held in abeyance.

Assistant Surgeon La Frenais has had further experience with his method of more thorough disinfection of the bites prior to giving the treatment. This appears to result in a substantial reduction in mortality from rabies. Statistical material on the usual lines is given in the appendices to this report.

The Indian Research Fund Association.—It is not fully realised in Bombay how much assistance to local research has been afforded by this progressive and far-seeing Association. During recent years the following sums of money have been contributed to this Institute or

to special problems in Bombay by the Government of India which finances the association:—

Name of Inquiry	1923-24	1924-25	1925-26	1926-27	1927-28	Total for five years
Plague research ...	5,100	9,000	39,694	45,844	76,364	1,76,002
Sprue and Bilharziasis ...	1,860	1,920	2,211	3,650	9,184	18,825
Biochemical ...	23,635	6,000	6,000	6,000	6,000	47,635
Pharmacological ...	.....	18,000	.....	.....	.....	18,000
Maternal Mortality and Morbidity in Child-birth.	.....	.....	5,000	14,915	17,270	37,185
Total ...	30,595	34,920	52,905	70,409	1,08,818	2,97,647

It will be noted that the two new units at the Haffkine Institute—Pharmacological and Biochemical—were both started, financed and mainly supported by the Association for three years before being presented in full running order to the Bombay Government. The generosity of the Association provides a means whereby promising young workers may be taken on and trained in research, some of whom are subsequently admitted as permanent members of the Medical Research Department. This portal of entry for young Indians is most valuable and the whole system is one that might well be followed by those wealthy citizens of Bombay who have at heart the higher education of their young scientists and the advancement of research into disease.

In addition to those amounts already given the Indian Research Fund Association has set aside a sum of Rs. 1,02,056 for researches into plague and maternal mortality for the year 1928-1929. At the same time it is a grave reflection on the citizens of Bombay that they should be content to leave the purely domestic problem of maternal and infantile mortality to outside agencies and to expect Government to finance such researches which in most other self-respecting countries are accepted as a matter for local responsibility.

The cold weather of 1927-28 was noteworthy for its visits of distinguished scientists from other countries, one of the chief objects of whose visit to Bombay was to inspect this Institute. On 21st December 1927 some 200 members of the Far Eastern Association of Tropical Medicine came to the Institute and spent the afternoon going through the different departments. Subsequently a conversazione was held in the Director's garden and the visitors expressed themselves well pleased with what they saw.

At a later date the members of the Health Interchange of the League of Nations paid a prolonged visit to the Institute. Opinions were freely expressed that the Calcutta School of Tropical Medicine and the Haffkine Institute were two of the most outstanding institutions in India, if not in the East. This comparison is satisfactory, especially in view of the fact that the former is well supported by the generosity of its citizens whereas this Institute struggles on understaffed and unassisted by any extra Government organization.

Hopes were raised that something might be done for this Institute as a result of the visit of Dr. Carter of the Rockefeller Foundation; and, with this in view, a comprehensive scheme was put up for the establishment of an Institute for Public Health Education and Research in connection with or adjoining this Institute. The scheme was well received by Dr. Carter, but it seems likely that Bombay is once more to suffer a disappointment, and for higher medical education must look elsewhere or go without.

More recently the Committee set up under the guidance of Sir Walter Fletcher to consider the reorganisation of research in India and the establishment of a Central Research Institute, visited this Institute and was presented with a scheme favouring the development of Bombay. It is too early to say if our hopes are to be realised or if the answer will once more be given "Heaven helps those who help themselves."

The boundary wall.—This improvement which had been recommended by successive Directors for the last decade or two was under consideration by Government, but the increasing number of contaminations of the vaccine made it desirable to expedite the process of consideration. The dust caused by the building operations in the vicinity and the fact that the corridors of the Institute had long been used as a caravanserai for men, goats, dogs, and bullocks, thereby giving rise to much dust and impurity, made it necessary to take immediate action. Government responded with alacrity and provided sufficient money to put up a boundary wall or wire fence of a temporary nature. A good proportion of the wall fell down a few weeks later, but was promptly re-erected, and now is in a state to deter all except those of abnormal agility. The barbed wire fence is a complete deterrent to cattle, somewhat less so to goats, none to dogs and little to men; so that it will be necessary in the near future to replace it by a wall.

The social life of the Institute.—The Critics Club:—This club came into existence in June 1924 at the instance of the Director and has for its members the medical and scientific staff of the Institute. Its objects are (1) to increase the social relations between the staff of the different departments, (2) to hold meetings when the researches carried on in different departments may be reviewed and criticised, and (3) to act as a stimulus for the junior members of the staff to undertake research work. The following subjects were discussed during the year:—(1) Review of researches carried on in the different departments of the Institute from April 1925 to November 1926—by Mackie; (2) Discussion on maternal mortality and the lines of investigation to be undertaken—by Balfour and Mackie; (3) Research on anti-plague vaccines—by Naidu and Jung; (4) The pathology and *post mortem* appearances in cases of sprue and pernicious anæmia—by Mackie and Chitre; (5) Subjects for discussion at the Conference of Research Workers at Calcutta—by members of the staff.

The special feature of these meetings is that it takes the form of a social, and not that of a formal, gathering. During the year a tennis tournament was organised for the staff who, with the members

of their family, contributed greatly to its success. A cricket club was also started this year for the staff; 19 matches were played—2 won, 3 lost, 8 drawn in opponents' favour, and 6 in the club's favour. Every effort is made to foster a democratic and sporting spirit among the members of the staff who number nearly 150.

The Institute has run during the year with a complete absence of friction, all the members of the staff working in coordination and cordiality one with another. This has made the administration of the Institute a real pleasure, and whatever credit accrues is due to the spirit of harmony and to the love of work for its own sake which has actuated the members of the staff.

Haffkine's Plague Prophylactic Vaccine

1. Manufacture.

Since the discovery of the prophylactic in Bombay in 1897 and its introduction as a measure of personal protection, the attitude of the public has undergone a profound change for the better; its efficacy as a protective measure is now universally recognised, and anti-plague inoculation is a popular measure in almost all districts. The demand for the vaccine, therefore, has steadily been on the increase.

From the opening of the laboratory to the end of 1927, 29,170,049 doses have been sent out. The output of the vaccine has been as follows:—

Year	Doses	Year	Doses
1897-1901	2,867,041	1912-1916	4,567,366
1902-1906	3,455,537	1917-1921	6,667,894
1907-1911	3,584,262	1922-1926	7,202,636

During the year 1927, the output was 825,310 doses; and the number of doses of anti-plague Vaccine despatched monthly during the past two years are detailed below:—

Month	1926	1927
January	176,676	75,012
February	348,948	88,350
March	252,987	65,050
April	280,250	49,878
May	236,235	2,676
June	123,024	8,556
July	13,800	18,720
August	64,722	46,596
September	163,224	119,136
October	216,180	87,808
November	450,356	133,850
December	88,980	129,678
Total number of doses ...	2,415,382	825,310

2. Distribution.

The geographical distribution of the vaccine in India and to places outside India during the years 1926 and 1927 is shown below:—

	1926	1927
British India—		
Bombay Presidency	97,251	73,330
Madras Presidency	102,326	117,216
Bengal, Behar and Assam	39,946	30,708
United Provinces of Agra and Oudh ...	228,281	102,660
The Punjab	1,185,880	8,148
North-West Frontier Provinces	28,195	3,012
Central Provinces and Berar	113,130	92,610
Delhi Province	4,250	702
Burma	65,106	115,332

		1926	1927
British India			
Indian States—			
Kathiawar	9,384		
Southern Maratha Country ...	2,376		
Savantwadi	1,116		
Baroda	870		
Palanpur	204		
Cutch	1,488		
Hyderabad	103,226		
Mysore	85,842		
Travancore	30,752		
Benares	252		
Patiala	8,016		
Jhind	2,070		
Faridkot	504		
Bhopal	300		
Jammu	10,200		
Jaipur	504		
Bikaneer	6,528		
Jodhpur	5,124		
	268,756	414,789	268,756
		.....	660
Portuguese Territory in India; *Countries outside India*—			
East Africa—Nairobi ...	54		
Arabia—Kamaran	1,008		
China—Foochow	504		
Japan—Shanghai	2,004		
Ceylon—Colombo	72		
Federated Malay States—			
Kualalampur	5,010		
Iraq—Baghdad	524		
Basrah	3,000		
	12,176	136,228	12,176
Total number of doses ...		2,415,382	825,310

3. Protection conferred by the prophylactic.

A. In Man.

Statistics of inoculation with the prophylactic.—The Indian Plague Commission of 1898-1899, from the statistics then available, concluded that "in every case, except that of Bulsar, inoculations had a considerable effect in warding off plague attacks from the inoculated, and that in every case, without exception, they rendered attacks among the inoculated less fatal than attacks among the un-inoculated." Since then statistics of

inoculation in man have been carefully collected and have been published from time to time. Most of these statistical data are summarised in the following tables :—

TABLE 1

Figures compiled from the Report of Bannerman (1900) and from the annual Reports of the Bombay Bacteriological Laboratory (1902-1916): —

—	Population	Number of attacks	Ratio per 100	Number of deaths	Ratio per 100	Percentage of survivors from attacks
Un-inoculated ...	3,227,842	59,668	1·84	50,735	1·5	14·9
Inoculated ...	1,937,213	4,126	0·21	1,620	0·08	60·7

TABLE 2

Figures taken from statistics of inoculation in the town of Ilkal, in Bijapur District, where a careful *house to house* inquiry was made; Report of the Bombay Bacteriological Laboratory for the years 1915-1916 :—

—	Population	Number of attacks	Ratio per 100	Number of deaths	Ratio per 100	Percentage of survivors from attacks
Un-inoculated ...	39	20	51·2	19	48·71	5·0
Inoculated ...	63	4	6·34	1	1·58	75·0

TABLE 3

Figures taken from the Annual Report of the Director of Public Health, Madras, 1926.

—	Population	Number of attacks	Ratio per 100	Number of deaths	Ratio per 100	Percentage of survivors from attacks
Un-inoculated ...	2,132	26	1·2	21	0·99	19·2
Inoculated ...	19,328	62	0·32	13	0·07	79·0

B. In Rats.

Since 1923, experiments (Morison, Naidu, Avari and Jung) have been carried out to estimate the protection afforded by the different brews of the prophylactic in experimental animals. For this purpose Madras rats, which are very highly susceptible to plague infection, have been immunised subcutaneously with 0·5 cc. (one-eighth the human dose) of the vaccine and after an interval of

7 days, these were infected with a dose of an emulsion of spleen of a rat which had died of acute plague, a dose found to kill nearly 100 per cent. of the infected rats within ten days. The protection conferred by the vaccine was measured by the number of survivors at the end of 15 days of infection. The results summarised below show that a high degree of protection is conferred on rats by the vaccine even within 7 days of inoculation against an infection with a large dose of highly virulent organisms. These results confirm the statistical evidence in man as to the value of the prophylactic against plague.

The average potency of the different brews of the prophylactic

Years	Number of brews examined	Number of experiments	Number of rats used	Survivors per cent. immunity	Controls	
					Rats used	Survivors per cent. immunity
1923-25 ...	29	49	1,015	33·9	482	6·7
1926 ...	62	82	3,350	29·8	360	6·6
1927 ...	48	72	3,470	36·4	338	5·4
Total ...	139	203	7,835	32·5	1,180	6·3

4. Variations in the potency of the prophylactic.

Since the commencement of the manufacture of the prophylactic in this laboratory, it has been observed that different brews varied in the degree of protection they conferred on man. For the production of a uniformly potent vaccine reliance was placed on the exact procedures used in its manufacture, such as, the use of a nutrient broth of nearly constant composition and the employment of highly virulent organisms to seed the culture medium, checked by animal experiment from time to time. Experiments of Naidu and Jung carried on different brews of the prophylactic showed that some brews, though manufactured under more or less identical conditions, yielded vaccines of very low potency. Therefore, every brew manufactured since 1926, has been tested for its potency on rats and only such brews which yielded a sufficient degree of protection have been issued for general use. It is believed that by this method of testing every brew the public are ensured of a supply of uniformly potent vaccine. The results of these experiments are here summarised.

Experiment on the potency of the different brews of the prophylactic.

	1926	1927
Total number of experiments	82	72
Number of rats used	3,710	3,808
Number of brews examined	62	48
Bad brews—below 20 per cent.	10	...
Poor brews—20 per cent. —30 per cent.	21	11
Good brews—30 per cent.—40 per cent.	17	17
Very good brews—above—40 per cent.	14	20

5. *Effect of temperature on the potency of the prophylactic.*—At the request of the Director of Public Health, Punjab, and on the lines outlined by him, experiments (Naidu and Jung) were carried out to determine the effect of the Punjab hot weather upon the potency of anti-plague vaccine stored at the Public Health Equipment Depôt, Jullunder. The brews examined were all sown by Germ No. 76, and incubated for 64 days. The vaccine was manufactured on the 17th January 1927, and its potency was tested on the 11th February 1927. Of the 29 rats immunised, 8 died of toxæmia within 7 days of immunisation giving a mortality of 27·5 per cent. The 21 survivors were infected with test dose of plague and the potency of the vaccine was estimated by the number of survivors after 15 days of infection; 11 rats out of the 21 died of plague, giving a per cent. immunity of 47·6; out of the 10 controls used in this experiment 9 died of plague with a percentage immunity (survivors) of 10·0.

The results of these experiments are summarised in a tabular form below and they show that within the range of temperatures recorded, the potency of this vaccine stored at Jullunder has not suffered adversely:—

Box No.	Brew No.	Average mean monthly temperature		Date of experiment	Rats used	Toxic deaths	Percentage	Survivors	Plague deaths	Percentage immunity (Survivors)
		Maximum	Minimum							
J. 1	65	77·2	73·9	13th May 1927 ...	60	8	13·3	52	23	55·7
H. 1	...	90·6	81·6	.....	60	12	20·0	48	22	54·1
...	...	...	...	.....	...	...	...	10	10	0·0 (Controls.)
J. 2	65	85·5	81·8	13th June 1927 ...	59	2	3·3	57	32	43·8
H. 2	...	87·1	85·1	.....	60	1	1·6	59	31	47·4
...	...	...	...	.....	...	...	...	10	10	0·0 (Controls.)
J. 3	66	90·3	87·8	8th July 1927 ...	59	2	3·3	57	31	45·6
H. 3	...	83·4	81·8	.....	60	3	5·0	57	33	42·1
...	...	...	...	.....	...	...	...	10	10	0·0 (Controls.)
J. 4	66	87·7	85·3	12th August 1927...	60	3	5·0	57	30	47·3
H. 4	...	83·8	82·3	.....	60	1	1·6	58	30	48·2
...	...	...	...	.....	...	...	...	10	9	10·0 (Controls.)
J. 5	73	84·4	82·8	6th September 1927.	59	5	8·4	53	23	56·6
H. 5	...	83·8	82·3	.....	60	3	5·0	57	31	45·6
...	...	...	...	.....	...	...	...	10	10	0·0 (Controls.)
J. 6	76	82·7	80·5	14th October 1927.	60	1	1·6	59	29	50·8
H. 6	...	85·1	83·2	.....	60	0	0·0	60	34	43·3
...	...	...	...	.....	...	...	...	10	9	10·0 (Controls.)
J. 7	76	75·7	73·2	11th November 1927.	59	4	6·7	55	18	67·2
H. 7	...	84·7	83·0	.....	57	5	8·7	52	17	67·3
...	...	...	...	.....	...	...	...	10	10	0·0 (Controls.)

6. *Examination of Rats for Plague in the City of Bombay.*—Plague is primarily an epizootic disease among rats, and man is its accidental victim due to his close association with this rodent. The prevention of plague, therefore, must very largely depend upon the destruction of the rat population. The very extensive examination of rats in Bombay was first undertaken in July 1905 by the Advisory

Committee for Plague Investigation in India to establish the correlation of plague rats with human cases. Since then systematic daily examination of rats, found dead or caught alive for the Health Officer of the city, has been carried out in this laboratory. This important work enables the Municipal Health Department to get a warning of an outbreak of plague and, as the locality whence each rat came is known, the locality where plague may be expected is foreshadowed. Since 1907, the annual mortality from plague in this city has been steadily on the decrease, and in 1926 only 56 deaths from plague have been recorded. It would, therefore, appear that this preventive measure has already had a beneficial influence on the mortality from plague in this city.

We understand that these examinations are the largest of the kind in any city in the world, and Bombay in this respect is frequently quoted as a model. Since July 1905 to the end of 1927 14,191,059 rats have been found dead or caught alive in this city; and of these 5,855,671 dead rats have been examined for plague. The results of these examinations are summarised in the following tables:—

Years	Total No. of rats found dead or caught alive	Dead rats examined *post mortem*	Plague infected rats	Percentage of infected rats	Human plague deaths
1902-06 ...	284,488	173,464	19,410	11·8	73,167
1907-11 ...	2,249,476	563,784	61,592	10·9	24,609
1912-16 ...	3,613,896	1,252,647	44,197	3·5	9,852
1917-21 ...	4,044,244	2,029,081	44,546	2·1	4,644
1922-26 ...	3,406,037	1,574,863	22,258	1·4	2,600

Rats received at the Institute from the Municipality during the last two years

Month	Alive	Dead	Total	Alive	Dead	Total
	1926			*1927*		
January ...	15,459	38,631	54,090	17,394	36,399	53,793
February ...	12,778	32,457	45,235	16,999	33,045	50,044
March ...	13,884	34,673	48,557	16,038	31,673	47,711
April ...	14,650	36,106	50,756	14,982	29,826	44,808
May ...	12,260	34,204	46,464	14,197	28,566	42,763
June ...	10,666	31,661	42,327	13,350	28,293	41,643
July ...	11,413	34,762	46,175	14,231	26,358	40,589
August ...	9,666	28,108	37,774	15,625	27,711	43,336
September ...	13,130	29,757	42,887	18,884	30,253	49,137
October ...	20,307	37,051	57,358	25,916	39,341	65,257
November ...	19,137	30,559	49,696	20,386	35,456	55,842
December ...	20,144	38,343	58,487	19,425	38,570	57,995
Total ...	173,494	406,312	579,806	207,427	385,491	592,918

Examination of dead rats for plague during the last two years

Month	1926				1927			
	Dead rats examined	Plague infected rats	*Rattus norvegicus*	*Rattus rattus*	Dead rats examined	Plague infected rats	*Rattus norvegicus*	*Rattus rattus*
January ...	27,985	85	76	9	26,443	75	71	4
February ...	23,780	121	106	15	25,186	97	95	2
March ...	25,925	235	199	36	21,540	130	117	13
April ...	23,726	261	221	40	19,539	173	158	15
May ...	24,496	198	173	25	18,900	258	220	38
June ...	21,028	91	79	12	17,754	180	156	24
July ...	26,006	78	67	11	19,020	126	100	26
August ...	18,478	79	70	9	18,765	77	69	8
September ...	19,881	50	45	5	20,806	68	56	12
October ...	26,595	37	24	13	16,995	59	50	9
November ...	17,331	13	12	1	27,710	53	42	11
December ...	28,267	38	36	2	29,174	125	101	24
Total ...	283,498	1,286	1,108	178	261,832	1,421	1,235	186

Relation of human plague deaths to plague deaths among rats, 1926-27

Months	1926				1927			
	Human plague deaths	Rats examined *post mortem*	Plague infected rats	Percentage mortality	Human plague deaths	Rats examined *post mortem*	Plague infected rats	Percentage mortality
January ...	2	27,985	85	0·3	2	26,443	75	0·3
February ...	9	23,780	121	0·5	8	25,186	97	0·4
March ...	4	25,925	235	0·9	17	21,540	130	0·6
April ...	10	23,726	261	1·1	54	19,539	173	0·9
May ...	11	24,496	198	0·8	73	18,900	258	1·4
June ...	5	21,028	91	0·4	19	17,754	180	1·0
July ...	2	26,006	78	0·3	16	19,020	126	0·7
August ...	3	18,478	79	0·4	9	18,765	77	0·4
September ...	4	19,881	50	0·3	4	20,806	68	0·3
October ...	3	26,595	37	0·14	4	16,995	59	0·3
November ...	0	17,331	13	0·07	0	27,710	53	0·2
December ...	3	28,267	38	0·13	1	29,174	125	0·4
Total ...	56	283,498	1,286	0·45	207	261,832	1,421	0·54

7. *Immunity of Bombay rats to plague.*—The fact that a large number of rats in India, where plague had occurred, were more or less immune has been noted by Simond and Walton ; and the Plague Research Commission (1906) found that 59 per cent. of Bombay rats withstood experimental infection by the cutaneous method. The estimation of the susceptibility of Bombay rats to subcutaneous infection was first carried out by Bannerman and Kapadia in 1904. They found that out of the 111 rats injected with broth cultures in doses of 0·2 cc. to 1 cc., 92·8 per cent. died of plague. The Commission (1906) also estimated the susceptibility of Bombay rats by subcutaneous injection of agar cultures in doses of 1/5th to 1/250th of an agar tube, and found that out of the 577 rats infected, 95·3 per cent. died of plague. These two observations are very similar and they indicate that Bombay rats were not more immune to plague in 1906 than they were in 1904. As the relative susceptibility or immunity of rats to plague infection is a matter of considerable importance in the epidemiology of this disease, the Commission (1910-12) . undertook a detailed study. They found that the employment of broth cultures was of little use for this purpose owing to the difficulty in regulating the number of plague bacilli injected so as to maintain an even dose in a long series of experiments and in maintaining the virulence of the organism. After a prolonged series of trials of different methods, they adopted the use of an emulsion of spleen of a rat which has died of acute plague for the test virus and a definite weight of spleen for the test dose. To obtain more or less constant results, it was necessary to inoculate an equal number of rats of constant and standard resistance along with rats whose immunity to plague it was proposed to measure. Subcutaneous injection of the test dose in rats caught in the city of Madras, a place which had practically escaped the ravages of plague and whose rats had not been exposed to infection, showed that these rats provided the necessary standard resistance. The Commission then estimated the resistance of rats of several places which have suffered almost continuously from plague, City of Bombay being one of them ; they concluded that the resistance of rats to plague is associated with the past prevalence of this disease, and is not due to any racial or local variation in susceptibility; they found that this resistance is not merely due to previous reception of sub-lethal and immunising doses of plague bacilli during the prevalence of an epidemic, but to the generation of a relatively immune progeny by the survivors of epidemics, and that the young

rats reproduced the resistance of their parents with remarkable fidelity. Since then the destruction of rats in this city has been continuously carried out on a very large scale and the human mortality has been on the decrease. Therefore, experiments (Naidu and Jung) were undertaken to estimate the relative immunity now enjoyed by Bombay rats, and the methods employed for this purpose are similar to those of the Commission. The results of these experiments with those of the Commission for Bombay rats are summarised for comparison in the following tables. It would appear that the relative immunity of Bombay rats to plague infection is on the increase:—

Experimental results on the relative immunity of Bombay rats to experimental infection with plague during 1926 and 1927

Month	1926				1927			
	Madras rats		Bombay rats		Madras rats		Bombay rats	
	Rattus rattus		*Rattus rattus*		*Rattus rattus*		*Rattus rattus*	
	Rats used	Percentage of plague deaths	Rats used	Percentage of plague deaths	Rats used	Percentage of plague deaths	Rats used	Percentage of plague deaths
January ...	...	...	...	...	50	90·0	116	18·2
February ...	...	...	...	...	50	94·0	119	19·4
March ...	...	...	...	...	60	95·0	120	27·5
April ...	...	...	...	...	50	92·0	99	19·2
May ...	20	90·0	50	26·0	50	100·0	120	21·7
June ...	30	93·4	50	16·0	40	97·5	119	16·9
July ...	40	90·0	49	6·2	30	96·7	120	14·2
August ...	30	96·7	200	6·0	40	97·5	120	10·0
September ...	30	93·4	149	20·9	50	100·0	120	10·9
October ...	110	99·1	113	35·1	30	93·4	118	27·2
November ...	100	94·0	117	18·9	20	100·0	119	22·7
December ...	110	95·5	120	21·0	50	96·0	117	10·3
Total ...	470	95·1	848	17·6	520	95·7	1,407	18·1

Experimental results of the Plague Research Commission, 1910-12.

onth	1910				1911	
	Madras rats		Bombay rats		Madras rats	
	Rats used	Percentage of plague deaths	Rats used	Percentage of plague deaths	Rats used	Percentage of plague deaths
January ...	100	93·0	100	45·0	200	99·0
February ...	.....	.....	.....	.....	200	98·5
March ...	.....	.....	.....	.....	100	98·0
April ...	.....	.....	.....	.....	100	99·0
May ...	12	100·0	48	39·0	100	95·0
June ...	200	95·5	200	49·5	100	100·0
July ...	.....	.....	.....	.....	.....	.....
August ...	100	98·0	100	47·0	100	99·0
September ...	100	97·0	100	62·0	100	95·0
October ...	.....	.....	.....	.....	.....	.....
November ...	100	100·0	100	73·0	100	96·0
December ...	.....	.....	.....	.....	.....	.....
Total ...	512	98·1	648	54·1	1,100	97·0

Month	1911—*contd.*		1912			
	Bombay rats		Madras rats		Bombay rats	
	Rats used	Percentage of plague deaths	Rats used	Percentage of plague deaths	Rats used	Percentage of plague deaths
January ...	200	54·0	100	98·0	100	24·0
February ...	200	51·5	.....	.....	.....	.....
March ...	100	36·0	.....	.....	.....	.....
April ...	100	23·0	100	97·0	100	27·0
May ...	100	19·0	90	97·0	100	21·0
June ...	100	45·0	.....	.....	.....	.....
July ...	.....	.....	.....	.....	.....	.....
August ...	100	21·0	.....	.....	.....	.....
September ...	100	16·0	.....	.....	.....	.....
October ...	100	30·0	.....	.....	.....	.....
November ...	100	42·0	.....	.....	.....	.....
December ...	.....	.....	.....	.....	.....	.....
Total ...	1,200	33·7	290	97·3	300	24·0

8. *Duration of immunity in Bombay rats following on the experimental infection with virulent plague bacilli.*—Experiments (Naidu and Jung) are in progress to study how long the immunity conferred on Bombay rats by experimental infection with virulent plague bacilli lasts. For this purpose, the survivors of the previous experiment on the susceptibility of Bombay rats to plague have been re-infected subcutaneously with the test dose at varying intervals, namely, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 days of the original infection. The results, so far, indicate that these animals are completely immune to fresh infection at the end of 180 days from the date of the original infection. These experiments are summarised below.

Duration of immunity in experimentally-infected Bombay rats :—

Date of first infection	Date of second infection	Interval in days	Survivors after 1st infection	Plague deaths	Survivors per cent. Immunity	Control	
						Madras rats	
						Rats used	Per cen Survivors
10th February 1927 ...	12th March 1927 ...	30	28	...	100·0	10	...
21st February 1927 ...	23rd March 1927 ...	30	50	...	100·0	10	...
10th March 1927 ...	10th May 1927 ...	60	32	...	100·0	9	...
22nd March 1927 ...	22nd May 1927 ...	60	49	...	100·0	10	...
11th April 1927 ...	12th July 1927 ...	90	48	1	98·0	10	...
21st April 1927 ...	21st July 1927 ...	90	26	...	100·0	10	...
10th May 1927 ...	8th September 1927.	120	44	...	100·0	10	10·0
11th June 1927 ...	9th November 1927 .	150	48	...	100·0	10	...
9th July 1927 ...	6th January 1928 ...	180	43	1	97·6	9	...

Pharmacological Unit

"The advisability of founding (in Bombay) a Pharmacological Laboratory or research institute in medicine for scientific experiment with and research into indigenous drugs" was considered for the first time in 1918. Several schemes were readily proposed and no less readily opposed; economy was necessary, and this scheme for research was to commence on modest lines. It took some years to find out lines modest enough, and it is only as late as 1924 that sanction was given to the establishment of a Pharmacological Laboratory at Parel. It soon became evident, however, that the scheme had been started on lines so very modest that the idea of a s parate and self contained institute could not be entertained, and the new venture had to figure modestly as one of the several units of the Haffkine Institute.

Started on very modest lines the Unit has continued its existence on the same very modest lines. Nevertheless the work done by this modest Unit has elicited from the Editor of the "*Lancet*" the remark that "The Indian Government may be congratulated on initiating such a line of research."

There cannot be two opinions as to the advisability of submitting Indigenous Drugs to a thorough scientific investigation. Modern science has to some extent pulled aside the veil of mystery that used to surround the therapeutic actions of drugs, and it is up to India to profit by whatever little progress has been made in that special branch of science. It is no longer permissible for India to advertise drugs on the mere strength of statements the truth of which no one has taken the trouble to confirm, or because of testimonials in the form of so-called clinical reports from uncritical or careless physicians. And it is inimical to the good name of India to continue booming medicines for no other reason than that they are swadeshi, even though their claims are out of harmony with accepted facts or are unsupported by worthwhile evidence. India is expected to fall into line with other nations in the field of drug therapy.

It is gratifying to note that some Indian Pharmaceutical Works of repute have at last realised the necessity of tests which should make it possible for trained chemists and pharmacologists to get an intelligent opinion of their products, to pronounce on their identity and purity, and to guard against adulteration or lack of strength. Their mistake, however, is to expect Government to bear alone and fully the expenses of the enterprise. The study of indigenous drugs is such a vast and difficult enquiry that it requires the collaboration of several expert research workers—chemists, pharmacologists, pharmacists, and clinicians—endowed not only with technical ability, but also with critical sense and disinterestedness. And such a study must be carried on in laboratories and hospitals adequately equipped for that special end. In fact, for the work to be successful, Government should not undertake to finance the institution without the assurance of good wish and pecuniary help on the part of wealthy and eminent Indians. "The strong spirit of Swadeshism" uught to manifest itself in the fostering of a spirit of unbiassed research and in the creation and maintenance of research centres.

But enough anent Indigenous Drugs, and one word about chemotherapy.

When salvarsan was introduced into medicine, now nearly twenty years ago, great expectations were formed regarding chemotherapy. The truth is that the practical successes have been so few and so very limited in their application that at the present time therapeutists are much more concerned with the contributions made by biology and biochemistry than with those attributable to the study of chemotherapy. "Fortunately, adds T. A. H. in Nature for the 18th February 1928, the contribution which chemotherapy makes to the welfare of humanity is not measured solely by these occasional therapeutical bull's eyes, but by the steady growth of systematised knowledge of the mode of action of drugs, which its study inevitably ensures, and this accretion of knowledge is just as likely to come from clinical failures as from clinical success..." It is in that spirit that the Pharmacological Unit have undertaken their investigation into the Chemotherapy of Bubonic Plague.

And now for a modest report of the work done during the year of grace 1927:—

I. Indigenous Drugs—

1. *Holarrhena antidysenterica.*—The results of a general study of this plant, dealing with its chemical composition and its pharmacological and therapeutic actions were published in an Indian Medical Research Memoir. "Results of real value to medicine, says the "*Lancet*" for the 7th January 1928, are likely to accrue from studies of Indian medicinal plants such as those undertaken by the authors of this memoir, and the Indian Government may be congratulated on initiating such a line of research."

2. *Gymnema sylvestre.*—The leaves do not contain any alcohol—or water—soluble substance which destroys glucose *in vitro*, nor do they yield any insulin-like matter. The so called *gymnemic acid* was isolated, and the pharmacological examination is being carried out.

3. A comparative study of the pharmacological properties of the Indian species of genus *Terminalia* is in progress.

4. *Garcinia mangostana.*—The therapeutic value of the fruit in the treatment of dysenteries is being determined.

II. Chemotherapy of Plague—

This inquiry was transferred to the Pharmacological Department early in December, and the synthesis of various compounds was immediately undertaken.

III. Publications—

1. *Caius, J. F., and Mhaskar, K. S.*—A study of Indian Medicinal Plants. *Holarrhena antidysenterica*, Wall. Indian Medical Research Memoirs. Memoir No. 6, May 1927.

2. *Caius, J. F., Naidu, B. P. B., and Shamsher Jung.*—The Bactericidal action of the Commoner Phenols and of some of their derivatives on *Bacillus pestis.* Indian Journal of Medical Research. XV, 1st July 1927.

3. *Caius, J. F., Kamat, S. A., and Naidu, B. P. B.*—The Bactericidal action of some organic compounds of mercury on *Bacillus pestis.* Indian Journal of Medical Research. XV, 2nd October 1927.

4. *Caius, J. F., and Naidu, B. P. B.*—Chemotherapy of Bubonic Plague. 7th Congress F. E. A. T. M., Calcutta, December 1927.

Biochemical Unit

1. Basal Metabolism.—The measurement of basal metabolism besides being of great value in the diagnosis and treatment of certain endocrine disturbances, has assumed fundamental importance in the study of some of the physiologic phenomena underlying nutrition. Therefore, during the year, the Unit was equipped with a chain compensated gasometer (a modification of Tissot gasometer) and Haldane Henderson apparatus for gas analysis. The members of the unit after spending some time in familiarising themselves with the particular apparatus acquired and the technique of basal

metabolic work generally, have commenced studying Normal Basal Metabolic Rate for adult Indians, with a view to see how it compares with the accepted standards for Europeans and Americans. More than 30 Indian males between the ages of 19-39 have been studied. The results so far obtained are interesting; the B. M. R. with a few exceptions is found to be 10 to 23 per cent. below the Du Bois Standards and correspondingly below Harris-Benedict Standards. It is proposed to collect a much larger number of data before drawing conclusions, but the low B. M. R. seems to be significant. The chief factors influencing B. M. R. were eliminated by adopting standard conditions for our experiments, *i.e.*, the determinations were made 12 to 14 hours after the evening meal, with the subject lying down in a state of complete repose. All the subjects were medical men and it was easy to get the necessary co-operation on their part. If the subsequent work confirms the preliminary figures, it will be our endeavour to find, if possible, the causes of this low rate. The work reported so far shows that race does not affect the rate, nor does the surrounding temperature if it be between 15° and 37° C. Could it be due to the state of nutrition of our subjects? We have noticed one significant fact in this connection: the level of protein metabolism of all our subjects was uniformly low, urinary excretion of nitrogen being between 5 and 7 grams per day.

2. *Sprue* (*a*) Pancreatic Function in Sprue—Originally the pale colour and bulkiness of sprue stools was attributed to defective biliary secretion leading to defective assimilation of fat. Actual estimation of bile pigments in the fæces showed that they were present in normal amounts. Later the excess of fat in sprue stools was considered to be due to defective pancreatic secretion. The work of various observers on this point has led to conflicting result and in our opinion this conflict is due to the methods employed in estimating the pancreatic secretion. Pratt and Spooner used Schmidt-Einhorn Thymus test and the Sahli glutoid salol capsules, while Brown measured the diastatic activity of urine and fæces. Since the modern duodenal tubes render possible a direct and more reliable examination of pancreatic ferments poured into the duodenum, we undertook to reinvestigate this point. We also undertook to analyse the fat content of fæces to see if fat composition of fæces would yield any definite information on the subject of pancreatic efficiency. Employing Saxon's Wet Method of fat analysis of fæces, we found that in only one of our series of seventeen cases of typical clinical sprue did neutral fat exceed 60 per cent. of the total fat content, while all cases, except one, showed normal splitting of fat. Quantitative estimation of diastase, trypsin, and lipase of the duodenal contents of five cases that we examined showed these ferments to be present in normal amounts. We have, therefore, come to the conclusion that the pancreas, as far as its external secretion is concerned, functions normally in sprue.

We also would like to mention that the total fat content of 14 out of our series of 17 sprue cases was high, ranging from 37·8 per cent. to 60·7 per cent. of the total dry matter. All our cases were on milk diet, it therefore occurred to us that high fat

content of fæces may have something to do with the milk consumption, considering that fat constitutes more than 50 per cent. of solids of milk other than sugar. We, therefore, examined the fæces of 17 bed cases on milk, and suffering from diseases other than sprue, *i.e.*, aneurysm of aorta, hemiplegia, rheumatic arthritis, etc. We found that the fat content of these cases ranged from 29·7 to 73·6 per cent. Neutral fat and split fat ratios agreed with similar ratios of sprue stools. As a result of these findings we think that high fat content of fæces in sprue does not disclose anything that is peculiar to sprue.

(*b*) *Liver Function in Sprue.*—This enquiry was taken up at the close of the last year to see whether the modern functional tests would throw any light on the efficiency of this organ, which is supposed to be affected in sprue. Thirteen cases of well marked sprue were studied, and all the cases were such as could be clinically described as definite cases of sprue. Emaciation, passage of bulky, frothy and pale stools, especially in the morning, involvement of tongue and anaemia were the diagnostic features. Function tests employed were (1) Levulose tolerance test, (2) The Van den Bergh Reaction, (3) Nitrogen partition of blood and (4) Bromsulphalein dye test of Rosenthal and White.

Nitrogen partition did not reveal any inefficiency. The bromsulphalein dye retention test also gave negative results, except in one case in which it may well have been due to extreme lowering of vitality generally on account of the approaching dissolution—the test was done three days before the patient died. Increase in the serum bilirubin in six cases, as shown by the indirect Van den Bergh reaction (0·8—3·0 units), indicated in our opinion increased destruction of erythrocytes rather than defective function of the liver. Seven cases gave abnormal levulose tolerance curves, the rise of blood sugar amounted from 21 mg. per cent. to 91 mg. per cent., and lasted for over three hours—some of these cases were reported last year. But on account of the non-specific nature of this test, and the negative results yielded by the other tests, we did not interpret our abnormal sugar curves in terms of liver inefficiency. In sprue inanition may more likely be responsible for disturbed carbohydrate metabolism. Recent work has shown that when a glucose (or levulose) tolerance test is done on an animal which has been fasted for four or five days, the rate at which the blood sugar level returns to normal is very much retarded, much more so than in an animal 4/5th of whose liver has been removed. From our work we concluded that the liver is not affected in sprue to such an extent as to show impairment by liver function tests.

3. *Nephrosis.*—Through the courtesy of Dr. J. P. Padshah of Goculdas Tejpal Hospital we were enabled to study five cases of this uncommon degenerative condition of the kidney. Four of these cases were of lipoid nephrosis, and one diabetic nephrosis. Cases of lipoid nephrosis were characterised by severe œdema, oligaemia, albuminuria, and changes in the protein and lipoids of the blood. In contradistinction to inflammatory condition of the kidney, all these cases gave normal urea concentration tests, blood pressure was not raised. Cholesterol content of blood varied from 230 mgs. per cent.

to 800 mgs per cent. (normal at 150—190) and fatty acids 454 mgs. per cent. to 1,760 mgs. per cent. (normal at 290—420). Two of these cases died, but *post mortem* examinations were not permitted. It is proposed to publish these cases with pathological findings as soon as a necropsy is made available.

4. *Anaemias.*—With a view to undertaking an intensive study of anaemias, a certain amount of preliminary work has been carried out to get standards for normal Haemoglobin and Cellular content of blood of healthy Indian adult males. The present methods of hæmoglobin determination used in clinical work were found unsatisfactory. In our study we determine haemoglobin content with Van Slyke constant volume blood gas apparatus and prepare fresh acid haematin standards and carry out colour comparisons in a colorimeter.

5. *Papers published.*—Liver Function in Sprue, Indian Journal Medical Research. 1928, XV, 3. Pancreatic Function in Sprue (in press).

Chemical Department

The routine work of this department consists principally in the examination of water, both chemically and bacteriologically, according to requirements of the public health authorities. Samples of milk, butter, and ghee are only occasionally received for examination.

Water:—Chemical Examination.—During the year under report ninety-seven samples of water were examined chemically. The sources from which they were obtained and the extremes of variations in total solids and chlorine are shown in Table I. The samples are grouped into three divisions, *viz.* well, bore, and surface waters, the latter including rivers, lakes, and tanks. The majority of the samples examined were unfiltered water.

Table I

Class	No. of samples	Parts per 100,000	
		Total solids	Chlorine
Surface water ...	59	5·0 to 44·0	0 to 6·6
Well water ...	23	30·0 to 156·0	1·2 to 41·0
Bore water ...	15	26·0 to 552·0	3·6 to 280·0

Of these samples only 19 were found to be chemically fit for potable purposes, when judged by the standards laid down in English text books on Hygiene.

There was one sample of water from a hot spring.

Bacteriological examination.—In the course of the year under report 31 samples of drinking water, 4 of swimming bath water, and several of effluents from Septic Tank, were examined bacteriologically. This examination consisted in determining (1) the total number of ærobic bacteria per cubic centimeter of the water, and (2) its least quantity fermenting MacConkey's bile-salt-lactose-neutral-red broth, with the productoin of acid and gas. The results obtained in the case of the 31 samples of drinking water are shown in Table II.

TABLE II

Source of water	No. of samples	Aerobic bacteria per c. c. varied from	Smallest quantity of water fermenting MacConkey's broth varied from
Wells	10 unfiltered	106 to 2,260	0·001 cc. to 0·1 cc.
River wells	4 „	420 to 11,000	0·01 to 1·0 cc.
Do.	1 filtered	50	10 cc.
Lakes	5 unfiltered	16 to 104	0·1 cc. to 10·0 cc.
Do.	2 filtered	2 to 6	10 cc.
Tanks	2 unfiltered	100 to 406	0·01 cc. to 1 cc.
Do.	3 filtered	33 to 121	10 cc.

In the case of the four samples from the Beach Candy Swimming Bath, two samples showed no lactose-fermenters in 10 cc. of the water, one showed in 1 cc. and one in 0·1 cc.; the number of ærobic organisms varied from 20 to 220 per cubic centimeter.

Fæcal Flora

The other work that is carried out in this department is in connection with the study of the fæcal flora. In the course of this study it was found that *B. alcaligenes*, a Gram-negative motile bacillus, which does not show any positive biochemical reaction was capable of producing nitrites from peptone, as determined by means of Ilosvay's reagent reported on in the Annual Report for 1925.

In anærobic work in connection with fæcal bacteria, Wright's method is the one that is generally used in small laboratories. Whenever observations regarding hæmolysis are to be made, blood agar slopes as ordinarily used are incapable of showing up this phenomenon except near the top of the slope where the medium is thin. In order that the whole length of the slope may be available, the simple procedure of superimposing agar slopes with a thin layer of five per cent. blood agar, was found to be very suitable both for anærobic and ærobic work. The superimposed layer of blood agar sets within a few minutes and the slopes are ready for use within about two hours of their being made. These superimposed blood

agar slopes, if properly made, preserve their bright red colour for more than a week, and are thus as readily available for work as the ordinary sloped media.

The above simple procedure is the outcome of Colonel Mackie's having laid special stress on the study of the hæmolytic characteristic of fæcal bacteria.

Antirabic Department

In last year's report attention had been drawn to the good results obtained by the preliminary cleansing and disinfection of wounds before commencing Antirabic Treatment. Now it has become a routine to open up and disinfect all wounds whether previously cauterized or not. The technique briefly is as follows :—

(*a*) A large percentage of people that are bitten usually come with some sort of a dressing on the wound the common dressings being cow-dung, chunam, or chilly powder. The first procedure is to remove these and cleanse the wounds and then introduce a probe to see the extent of damage done. Having satisfied that the wounds whether single or multiple, punctured, or lacerated, require opening up, proceed with a pair of sharp scissors or curved bistoury to open up and wash the wound thoroughly with 2 per cent. acriflavine in alcohol and plug the wound with a piece of gauze soaked in the above solution. This dressing is applied for the first 48 hours and then ordinary eusol or carbolic dressing is substituted till the wounds heal. If a general anæsthetic is required it should be given.

(*b*) This procedure is applied to all wounds whether septic or not. If œdema is present hot saline fomentations should be applied frequently till the swelling subsides, and if the patient requires hospital treatment he ought to be sent to a hospital and the above line of treatment carried out. The antirabic injections being given by the hospital authorities.

(*c*) No attempt to stitch the wounds should be made, even if it has to cause disfigurement. Out of 729 persons who underwent the full course of treatment, the above line of treatment was applied to 636 and only one developed rabies and died. Of the remaining 93, *i.e.*, those on whom the above method was not done, 4 died.

Antirabic treatment.—The number of patients who received the full course of treatment in this laboratory during the year 1927 was 729, an increase of 81 on the number treated during the previous year 1926. There were in addition 147 persons who for various reasons did not complete the full course of treatment, and 505 persons who were given advice but no treatment.

No patient died during the course of treatment, one patient died within less than fifteen days after the completion, and four died more than fifteen days after the completion of treatment. Of the above five deaths, one was from among those who discontinued treatment.

During the year 4,302 treatments were issued to out-centres. The statement of cases treated at the different centres during 1927 is published in Table VIII. Seventy-two treatments were sent to the various veterinary surgeons for treating dogs and other animals that were bitten by rabid animals.

Examination of brains for rabies.—The following is the statement of the examination of brains of dogs and other animals for the diagnosis of rabies during the year 1927 :—

Bombay—		
Through the Veterinary College	...	82
Through the Bombay Police	...	15
Other places in Bombay	...	32
Out Stations	...	92
Total	...	221

The result of examination of these animals was as follows :—

Received in a condition unfit for examination	11	4·97	per cent.
No evidence of rabies	67	30·31	,,
Negri bodies found, *i.e.*, brains obtained from rabid dogs	143	64·72	,,
Total	221		

Vaccine Department

Plague—

4,636 brews of 1,000 cc. each were decanted.

169,493 bottles (approximately) were filled with vaccine.

11,528 bottles were tested for sterility.

1,065,000 doses of the prophylactic were in all sent to the Despatch Department for packing.

Antirabic vaccine—

388 brews were decanted.

45,397 bottles were filled :—

5,612	bottles of 25 ccs.
6,326	,, 10 ccs.
33,459	,, 5 ccs.
45,397	

882 bottles were tested for sterility.

74,171 doses or 5,298 treatments were sent to Despatch Department.

Other vaccines prepared—

5,360 ccs. Mixed T.A.B. vaccine.

30,790 ccs. Cholera vaccine.

Dead emulsions prepared—

650 ccs. Typhoid.
450 „ Paratyphoid A
400 „ „ B
250 „ „ C
250 „ *B. proteus.*
250 „ *Micrococcus melitensis.*

Stock cultures—

265 various stock cultures were sub-cultured at least once a month.

13 Special cultures of *B. pneumococci* were sub-cultured on special media every four days. (Blood media were prepared from time to time for the above cultures.)

Miscellaneous work—

Decanting—

2 flasks of bile peptone glucose.
4 „ carbolized saline.
6 „ ox bile.
1 „ normal saline.
3 „ citrate saline.
1 „ bile peptone glycerine.
3 „ horse serum.
85 „ cholera vaccine.
8 „ T.A.B. „
3 „ dead emulsion of Typhoid.
1 „ „ „ Para A.
1 „ „ „ Para B.
1 „ *Proteus.*
2 „ *Micrococcus melitensis.*
2 „ Quinine.

Testing for sterility—

266 bottles of various vaccines, etc.

Inoculation—

101 persons were inoculated against plague.

Articles supplied—

	No. of indents	Quantity
Vaccine—		
Mixed T.A.B.	84	606 cc.
Cholera	18	410 cc.

	No. of indents	Quantity
Dead emulsions—		
Typhoid ...	27	201 cc.
Paratyphoid A ...	22	146 cc.
„ B ...	22	146
B. coli ...	1	2 cc.
M.M. ...	2	12 cc.

A large number of stock cultures of living organisms were supplied to outside workers.

Snake venom.—Six indents were complied with for Cobra and Daboia venom.

Snakes.—Twenty-one Cobras, 17 Russell's Vipers, 6 Kraits were received during the year. On an average 9 Cobras and 18 Russell's Vipers were taken out fortnightly for extraction of venom and feeding.

GENERAL DIAGNOSTIC DEPARTMENT

	+	−	Total
Blood smears for malaria ...	32	95	127
„ „ rat bite fever	....	5	5
„ „ microfilaria	5	4	9
„ „ leucocyte count	...	...	125
„ „ cultures for plague	48	1	49
„ „ other organisms (Typh.)	2	10	12
Blood for Widal for T.A.B.	57	79	136
„ „ M.M.	1	3	4
„ „ Weil Felix	....	1	1
„ Wassermann	646	904	1,550
„ Sach's Georgi	572	978	1,550
„ Formol Gel	21	32	53
„ Groupings	...	...	46
„ Hæmoglobin and total	...	...	2
Autogenous vaccines	...	...	177
Sputum for T.B.	30	55	85
„ other organisms	...	...	85
Scrapings for leprosy	32	28	60
Swabs for diphtheria smears	2	23	25
„ „ cultures	2	23	25
Smears for gonococci	21	6	27
„ B. pestis	12	10	22
Urine for general analysis	...	...	133
„ bacteriological examination	...	...	53
Fæces for ova	...	...	143
„ cultures	...	...	117

	+	−	Total
Cultures identified	...	...	126
Pus for cultures, sputum, urine	...	...	78
C. S. for meningococcus and cultures	...	21	21
C. S. for T. B	...	21	21
Snakes identified	...	...	28
Snake bites treated or advised	...	...	24
Disinfectants tested	...	...	10
Microscopes examined	...	...	14
Salvarsan injections given	...	...	58
Animal experiments	...	...	20
Scrapings for *treponema pallida*	...	3	3
Pleuritic effusion for T. B.	2	3	5
Fæces for cholera	65	4	69
Agar sample tested	...	...	1
Blood smears of hounds for trypanosomes	44	21	65
Blood smears for Piroplasma	1	6	7
,, ,, Anthrax	...	3	3
,, ,, L. D. Bodies	...	2	2
Colloidal gold test	...	...	1
Globulin estimation	...	...	7
Water samples for cholera vibrios	...	2	2
Peritoneal fluids for T. B., etc.	...	1	1
Semen for spermatozoa	...	...	3
Surra (blood smears of horses)	16	1	17
			5,207

1,550 bloods were examined for Wassermann and Sach's Georgi reactions. The Wassermann reaction was positive in 646, whereas Sach's Georgi showed only 572, *i.e.*, Wassermann reaction was more sensitive in 11·4 per cent. of cases.

Work on bacteriophage was carried on throughout the year and strains of phages for Gonococcus, *B. pestis*, *B. dysenteriæ*, cholera and *B. coli*, have been isolated.

Pathological Section

Pathological tumours received and examined during the year 1927 :—

Non-malignant	90
Malignant	68
Granulomata	16
Other pathological conditions	136
Total ...	310

Table showing analysis of the malignant tumours and their distribution in the body :—

Part of Body	Carcinoma	Sarcoma	Endothelioma	Melanoma	Rodent ulcers	Total
Anus	1	1	...	...	...	2
Ankle	1	...	...	...	...	1
Breast	11	1	...	...	...	12
Bladder	2	...	...	...	...	2
Back	1	...	...	...	...	1
Cheek	1	...	...	...	...	1
Cervix	3	...	...	...	...	3
Ear	...	...	...	...	1	1
Eyeball	...	1	...	...	...	1
Foot	...	1	...	...	...	1
Face	...	1	...	...	...	1
Gland	4	...	1	...	...	5
Heel	...	...	...	1	...	1
Ingl. Region ...	1	...	1	...	...	2
Jaw	...	...	...	1	...	1
Kidney	1	...	...	...	...	1
Maxilla	...	...	1	1	...	2
Nose	2	2	...	...	...	4
Neck	...	...	5	...	...	5
Ovary	1	...	...	...	...	1
Orbit	...	1	...	...	...	1
Prostate	1	...	...	...	...	1
Penis	2	...	...	...	...	2
Uterus	5	...	...	...	...	5
Scalp	2	...	...	...	...	2
Scrotum	...	2	...	...	...	2
Stomach	...	1	...	...	...	1
Thigh	1	1	...	...	...	2
Tongue	1	...	...	...	...	1
Testis	...	1	...	...	...	1
Tibia	...	1	...	...	...	1
Vagina	1	...	...	...	...	1
Total ...	42	14	8	3	1	68

Special Researches Financed by the Indian Research Fund Association

The *Plague Researches* which come under this category have already been reviewed.

The *Schistosome Enquiry* which was started by Dr. Hamilton Fairley has been continued throughout the year by Jasudasan and Mackie on the lines laid down in correspondence with Dr. Fairley in Melbourne. These researches will be closed down on March 31st 1928, and the large volume of work which has resulted will be published in due course. A brief note on the subject will be found later in the report.

The *Enquiry on Maternal Morbidity and Mortality in Childbirth* has been carried on by Drs. Miss Balfour, Miss Mehta and Madame Emanuelov. The progress of this enquiry is the subject of a section which will also be found later in this report.

Researches on Sprue.—This disease was taken up first in 1923 and a comprehensive study was begun on the lines laid down by Fairley and Mackie. It was their idea to take one disease and to make as thorough a study of its natural history as possible; and sprue, as a prevalent and serious disease in Bombay, was selected for investigation. The work was interrupted in 1925 by the departure on leave of the two principal investigators and was resumed in November 1926 on the return from leave of the latter. Unfortunately for the progress of the investigation, Fairley himself developed a severe attack of sprue and left in the summer of 1925, and subsequently had to sever his connection with India. The sprue enquiry was reopened in November 1926 and has been carried on since that date till March 1928 when the transfer of Mackie to Simla necessitated its being again closed down.

This enquiry has been conducted on broad lines and, as the list of publications shows, a number of workers in the different departments of the institute have taken part in the work. A very brief summary of their broad conclusions may be given as follows:—

(1) *M. psilosis* (Ashford) is found in the majority of cases of sprue, but is present also in similar proportions in cases of diarrhœa not sprue and in healthy persons.

(2) *M. psilosis* is toxic to rabbits by intravenous inoculation producing focal nephritis and death. It is less toxic by other routes and generally produces localised abscesses.

(3) The study of the hepatic function by lævulose tolerance and bromsulphalein dye tests does not bear out the contention that the function of the liver is seriously at fault.

(4) The study of the intestinal digestive juices indicates that the fat protein and sugar splitting enzymes are acting normally. Any serious derangement of the pancreas is therefore unlikely.

(5) The study of the morbid anatomy and histology of sprue does not reveal any pathognomic lesions. A general condition of aplasia with probable loss of absorptive power is found in the small intestine but it is difficult to say whether this is the cause or the effect of the disease.

(6) The blood in sprue shows changes of an aplastic type which confirms the condition of the bone marrow. The blood picture is different from that seen in pernicious anæmia.

(7) No bacillus peculiar to sprue has been found. A vibrio-like organism has been recovered from the duodenal contents and the blood of three cases, but its significance is not known. Hæmolytic bacteria are commonly found in sprue stools, a fact which may throw some light on the anæmia.

(8) Sprue is regarded as a clinical entity quite distinct from pernicious anæmia. The following reasons may be adduced :—

(*a*) In sprue the patient is progressively and profoundly emaciated.

(*b*) Achlorhydria, though sometimes present, is not invariable.

(*c*) The blood picture differs from that of pernicious anæmia.

(*d*) The bone marrow is generally aplastic.

(*e*) Spinal symptoms are rarely if ever seen in sprue.

(*f*) Recovery is frequent in sprue, but rarely or never occurs in pernicious anæmia.

(*g*) The clinical picture of the two diseases is markedly different, and so are the lines of successful treatment.

List of publications on Sprue in the press or shortly to be published:—

1. Yeasts and Sprue; by Mackie, F. P., and Chitre, G. D.

2. Animal Experiments and Sprue; by Mackie, F. P., and Chitre, G. D.

3. The Association of Bowel Disease with Vitamin C Deficiency; by Mackie, F. P., and Chitre, G. D.

4. The Morbid Anatomy of Sprue; by Mackie, F. P., and Fairley, N. H.

5. The Microscopical Changes occurring in Organs after death; by Mackie, F. P.

6. The Liver Function in Sprue; by Sokhey, S. S., Gokhale, S. K., Malandkar, M. A., and Billimoria, H. S.

7. The Pancreatic Function in Sprue; by Sokhey, S. S., and Malandkar, M. A.

8. The Bacteriology of Sprue; by Mackie, F. P., Gore, S. N., and Wadia, J. H.

9. Note on the presence of an undetermined organism in three cases of Sprue; by Mackie, F. P., and Gore, S. N.

10. The Blood in Sprue; by Mackie, F. P., Fairley, N. H., and Billimoria, H. S.

11. The Serology of Sprue; by Fairley, N. H., and Jasudasan, F.

12. The Clinical Aspect of Sprue; by Fairley, N. H., and Mackie, F. P.

13. The Treatment of Sprue; by Fairley, N. H.

Anti-plague Inquiry

1. *Standardisation of the prophylactic.*—Following on the manufacture of the prophylactic, Haffkine (1897) attempted to standardise the vaccine by its opacity combined with the degree of reaction it set up in man. Bannerman (1905) found that the methods of standardising the different brews by (*a*) the febrile reaction produced, (*b*) the opacity test, and (*c*) the amount of alkali present in the vaccine were all wanting. During the same year Liston by a modification of Wright's method of counting the number of organisms in a given culture fixed as a suitable standards "the maximum quantity of a vaccine which will in 48 hours protect a guineapig weighing 250 to 300 grammes against at least 109 times the lethal dose of a plague

culture isolated from the body of an animal not more than one week previously." Until recently standardisation of the prophylactic consisted in the exact procedures employed during its manufacture.

The usual methods of standardising vaccines are inadmissible in the case of the prophylactic because the culture medium during the long period of incubation becomes highly alkaline resulting in the lysis of most of the bacilli; and experiments of Morison, Naidu and Avari (1924) have shown that the immunising agent in the finished product is the clear supernatant fluid and not its sediment. Experiments carried out since 1923 to study the factors which have an influence on the potency of the prophylactic suggested the possibility of standardising the brews of the prophylactic by animal test. In view of the variations in the potency of different brews it seemed necessary to ensure the supply of a fairly uniform and potent vaccine for general use.

In addition to the experiments on the potency of the different brews of the prophylactic manufactured here, experiments to determine the smallest amount of plague-spleen which would produce a uniform mortality of 90 to 100 per cent. in test animals were carried out with 1,590 rats, 180 guineapigs, and 30 rabbits, in doses varying from 0·003 mgm. to 0·0,000,000,000,000,000,000,000,003 mgm.; and in these doses no appreciable influence on the mortality from plague in these animals was noticeable within 15 days of infection; with smaller doses in some cases the mortality (daily) tended to spread over a slightly longer period. In view of these results, experiments were also carried out to compare the relative mortality following on the cutaneous method of infection with the subcutaneous infection of plague-spleen in dose of 0·003 mgm. in rats. The results showed that out of the 1,278 rats cutaneously infected the percentage mortality from plague at the end of 15 days of infection was 94·5, while of the 920 rats sub-cutaneously infected with 0·003 mgm. plague-spleen, 867 died of plague with a percentage mortality of 94·2 in 15 days of infection.

Nine experiments, using 325 rats, have been carried out to study the immunising value of smaller doses of the prophylactic, and eighteen experiments, using 198 guineapigs, have also been carried out to study the possibility of using guineapigs in preference to rats for estimating the toxicity and potency of the prophylactic. These experiments are still in progress, but the results so far seem to indicate that the results obtained with guineapigs are very variable, while with rats more uniform and comparative results are obtained.

2. *Preparation of a potent anti-plague serum.*—The anti-plague serum of the Pasteur Institute, Paris, is the one now largely used in the treatment of plague and the results obtained from its use have varied considerably in different places. Yersin (1897) was the first to employ anti-plague serum in Bombay. Since then, anti-plague sera prepared by Roux (Pasteur Institute, Paris), Haffkine (Bombay) Lustig (Florence), Terni (Messina), Brazil (San Paolo, Brazil), and Rowland (Lister Institute, Elstree) have all been tried in the Municipal Plague Hospitals, Bombay. It has been found here that the results obtained from sera prepared by Roux, Lustig, and

Rowland have been more or less the same, and that their use in hospital cases reduced the plague mortality by 10 to 12 per cent. All these sera have been obtained from the immunisation of horses, either by the injection of plague cultures or their nucleoprotein. Haffkine (1897) found that among the cases treated at Poona with sera prepared from horses, cattle, and sheep, those patients who were treated with sheep's serum were the only ones who seemed to have been benefitted by treatment.

D'Herelle (1926) found that the local strains of plague bacilli isolated in India were very virulent and resistant to the lytic action of his antipestiphage employed in Alexandria for the treatment of plague and, therefore, recommended the use of local virulent strains for the preparation of anti-pestiphage. Experiments by Naidu, Malone, and Avari, carried out here, showed that vaccines prepared from virulent cultures were more potent than those prepared from avirulent cultures.

Therefore, experiments (Naidu and Jung) are in progress to study the factors which may have an influence in the production of a potent serum. During this year, 55 rabbits were immunised with very virulent cultures of plague bacilli isolated from the heart blood of rats suffering from acute plague. Injections were made at weekly intervals and agglutination tests were carried out on the sera at intervals of 6 weeks. The results of these experiments showed that (1) subcutaneous injections with killed cultures followed by living cultures yielded sera of low agglutinating titre (1-200 to 1-800), (2) intravenous injections with dead cultures followed by living ones yielded sera of high agglutinating titre (1-16,000 to 1-32,000) and (3) some rabbits continued to yield sera of low agglutinating titre in spite of repeated injections over a long period.

Twelve experiments, involving the use of 28 rabbits, were carried out to determine the minimum dose of spleen of a rat which has died of acute plague, which when injected sub-cutaneously would kill nearly 100 per cent. of rabbits.

With this dose, rabbits experimentally infected with plague were treated with immune rabbit serum in varying amounts. For this purpose a dose of 2 cc. of the serum was injected intravenously ; and when this dose was repeated, it was at intervals of 24 hours. Some of the infected rabbits were treated within an hour of infection, while others were treated at intervals of 24, 48, 72, 96, 120, 144 and 168 hours after infection. The results of 7 experiments, using 148 infected rabbits, showed that the total mortality from plague among the treated rabbits was 18·9, while the mortality among the controls was 100 per cent., and that out of 95 rabbits in whom treatment was commenced 48 hours after infection the mortality per cent. was 26·3. There were no deaths from plague after the 9th day of infection either among the treated or the controls. Findings also seemed to indicate that better results were obtained with sera of high agglutinating titre, and that it is possible to save an infected animal from death even on the 4th or 5th day of infection with a highly agglutinating serum. These experiments are being repeated.

3. *Chemotherapy of plague.*—One hundred and fourteen experiments were carried out on 32 organic compounds prepared by Kamat and Caius, to study the bactericidal properties of these compounds on *B. pestis* in vitro. One of these compounds, namely, mercurated trypan blue, was highly bactericidal to *B. pestis* as compared with mercurochrome 220 soluble. Therefore, 10 experiments involving the use of 400 rats and 25 rabbits were carried out to estimate the toxicity of this compound of mercury. Rats were inoculated subcutaneously and rabbits interavenously in single and in repeated doses with varying amounts from 0·6 mgms. to 9·0 mgm. for rats and from 5 mgms. to 10 mgms. for rabbits. The results showed that a dose of 5·4 mgm. for rats and 9 mgms. for rabbits was lethal. Repeated doses of 6 mgms. on 5 consecutive days had no toxic effect for rabbits. Then, the therapeutic value of this compound in the treatment of plague was estimated on rabbits along with mercurochrome-220 soluble for purposes of comparison. Rabbits weighing from 1120 to 2000 grammes were infected subcutaneously with a millionth of the test dose employed for rats (0·003 mgm. spleen of a rat which has died of acute plague); and the treatment was commenced immediately after infection, a dose of 5 mgms. being administered intravenously; when this dose was repeated it was at intervals of 24 hours. The total amount administered varied from 5 mgms. to 30 mgms. The results of these experiments, summarised below, showed that these organic compounds of mercury had little influence on *B. pestis* in vivo, although the rabbits were infected with a comparatively small dose of the test virus and the treatment was commenced immediately after with intravenous injection of varying amounts of these compounds from 5 mgms. to 30 mgms., *B. pestis* was found in every fatal case:—

Mercurochrome-220 soluble				Mercurated trypan blue compound			
Weight in grammes	Total amount mgms.	Death in days after infection	Survivors at the end of 30 days	Weight in grammes	Total amount mgms.	Death in days after infection	Survivors at the end of 30 days
2,000	5	10	...	1,900	5	4	...
1,120	5	...	1	1,340	5	3	...
1,300	10	7	...	1,780	10	...	1
1,230	10	7	...	1,340	10	5	...
1,940	15	7	...	1,780	15	8	...
1,280	15	8	...	1,340	15	...	1
1,920	20	10	...	1,770	20	4	...
1,280	20	7	...	1,380	20	9	...
1,900	25	6	...	1,770	25	7	...
1,300	25	6	...	1,650	20	4	...
1,650	15	3	...	1,770	30	6	...
2,000	30	...	1	1,350	30	6	...
Control				Control			
1,900	...	7	...	1,500	...	8	...

4. *Bacteriophage in the treatment of plague.*—The publication of the successful treatment of four cases of bubonic plague at Alexandria by d'Herelle with anti-pestiphage was brought to the notice of the Government of India who arranged for a supply of this phage and Avari and Naidu were detailed to carry out the clinical test on a fairly large series of cases of plague. After the arrival of the bacteriophage in March 1926, treatment with this phage was carried out in Hyderabad (Deccan) and in Agra. The results of treatment are summarised below:—

Bacteriological diagnosis		Treated cases		Control cases		Remarks
Bubo	Blood	No.	Deaths	No.	Deaths	
+	+++	29	29	29	29	Septicæmia, severe.
-	+++	3	3	6	6	
+	++	4	4	3	3	Septicæmia, moderate.
-	++	2	2	3	3	
...	...	38	38	41	41	
+	+	14	11	9	6	Septicæmia, slight.
-	+	4	4	2	1	
...	...	18	15	11	7	
+	-	40	12	29	6	Purely bubonic.
-	-	7	1	16	9	Clinically plague.
...	...	103	66 (64 per cent.)	97	63 (65 per cent.)	Total number of cases studied.

These results pointed to the conclusion that septicæmic cases of moderate and severe degree do not recover, whether treated with bacteriophage or not, and that treatment with bacteriophage, even in cases of slight degree of septicæmia and in cases of purely bubonic type, does not influence the course of the disease and the percentage mortality; in many of these cases the bacteriophage was administered in larger doses than those recommended, and in some the bacteriophage was administered intravenously.

At Agra, Avari and Naidu estimated the potency of the bacteriophage and found that the addition of the bacteriophage in a dose of 1 cc. to a broth culture of plague bacilli produced lysis of the organisms in 24 hours. At this Laboratory Morison and Jung estimated the curative value of this bacteriophage on experimentally

infected rats and found no evidence that the bacteriophage exerted any curative influence. While these tests were nearing completion Dr. d'Herelle arrived in Bombay; and in view of these disappointing results, he recommended "that researches be made in India with the object of obtaining a bacteriophage able to produce complete lysis of a 24 hours' virulent culture of *B. pestis* (local strains) at a temperature of 38° to 40°C. within the shortest time of contact (4 to 6 hours)". "As bacteriophage virulent for *B. pestis* is found in the intestinal contents of rats which resist infection with plague and also in the intestine and in the buboes of men during recovery a virulent bacteriophage should be isolated from these sources; but of the two the human source seems preferable". Since then Avari has carried out an extensive series of experiments to isolate a potent bacteriophage against a culture of plague. For this purpose he employed "Martin's broth" at a hydrogen-ion concentration of pH 7·5 as recommended by d'Herelle. The bacteriophage was sought in the buboes, spleens, livers, supra-renals, kidneys, hearts, lungs, cervical gland, brains in addition to the fæces of rats which survived an acute infection after an experimental dose of *B. pestis*. One hundred and thirty-eight rats have been thus examined. The discharge from buboes of 3 plague patients and the fæces of 12 such patients have also been examined. Bacteriophage of varying potency was found 10 times in the 1,242 examinations carried out. Avari also found that the best time to look for the phage in experimentally infected animals was on the 8th or the 9th day of infection. He has now isolated a bacteriophage from buboes and spleens of rats which brings about the lysis of a plague culture in broth in one and half hours to two hours; when this phage is diluted to 1 in 1,000 lysis occurs in three and half hours; lysis occurs in 6 hours when it is diluted to 1 in 10,000 and in 24 hours in a dilution of 100,000. The results of these experiments are summarised in the following two tables:—

Table showing optimum period and the organs from which the phage was obtained

Days after infection with *B. pestis*	Bubo		Spleen		Liver		Supra-renals		Kidneys	
	+	—	+	—	+	—	+	—	+	—
8th day	6	15	5	16	1	20	...	21	...	21
9th day	3	6	2	7	1	8	...	9	...	9
10th day	...	1	1	...	...	1	...	1	...	1
15th day	1	106	...	107	...	107	...	107	...	107
Total rats ...	10	128	8	130	2	136	...	138	...	138
Human cases ...	...	3	...	...	...	...	...	...	...	...

Days after infection with *B. pestis*	Cervical glands		Hearts and lungs		Brain		Fæces	
	+	—	+	—	+	—	+	—
8th day	...	21	...	21	...	...	...	21
9th day	...	9	...	9	...	9	1	8
10th day	...	1	...	1	...	1	...	1
15th day	...	107	...	107	...	107	...	107
Total rats ...	...	138	...	138	...	117	1	137
Human cases ...	...	...	...	...	...	...	...	12

+ = Phage found; — = Phage not found.

Table showing the potency of phages obtained

Phage lysing *B. pestis* in	Buboes	Spleens	Livers	Suprarenals	Kidneys	Cervical glands	Heart and lungs	Brain	Fæces
2 hours	4	2	...	...	...	...	...	...	...
4 „	3	3	...	...	...	...	...	...	...
6 „	1	1	1	...	...	...	...	...	...
9 „	1	2	...	...	...	...	...	...	...
12 „	1	...	1	...	...	...	...	...	...
13 to 24 hours ...	...	...	...	...	...	...	...	...	...

Experiments are now in progress to estimate the curative value of this highly potent bacteriophage in experimentally infected animals.

List of Publications on Plague from the Haffkine Institute

The Indian Journal of Medical Research.

1924. The production of immunity against plague by vaccine.

1. Haffkine's plague prophylactic—Morison, Naidu and Avari.
2. Agar cultures—Morison, Naidu and Avari.
3. Immunisation by the oral administration of plague vaccine with and without bile—Morison, Naidu and Avari; (Vol. XII, No. 2).
4. A commentary on the foregoing papers—Mackie.

1925. The bactericidal power of the blood of rats as a measure of their immunity to plague—Malone, Avari and Naidu; (Vol. XIII, No. 1).

1926. Notes on the potency of Haffkine's plague prophylactic—Naidu, Malone and Avari; (Vol. XIII, No. 4).

The treatment of plague with mercurochrome-220 soluble—Naidu and Shamsher Jung; (Vol. XIV, No. 2).

The toxicity and immunising value of sensitised anti-plague vaccines—Naidu and Shamsher Jung; (Vol. XIV, No. 2).

The value of the thermoprecipitin reaction in the diagnosis of rats putrefied and died of plague—F. M. Marras; (Vol. XIV, No. 2).

A rapid method for the diagnosis of plague—F. M. Marras; (Vol. XIV, No. 2).

1927. A note on the nutrient broth now used for the culture of *Bacillus pestis* and its hydrogen-ion concentration—Naidu and Shamsher Jung; (Vol. XV, No. 1).

The bactericidal action of the commoner phenols and of some of their derivatives on *Bacillus pestis*—Caius, Naidu and Jung; (Vol. XV, No. 1).

Production of alkalinity by *B. pestis* in broth and the effect of this alkalinity on the toxicity and potency of the prophylactic—Naidu and Shamsher Jung; (Vol. XV, No. 2).

The bactericidal action of some organic compounds of mercury on *Bacillus pestis*—Caius, Kamat and Naidu; (Vol. XV, No. 2).

Papers read at the 7th Congress of the Far Eastern Association of Tropical Medicine, Calcutta, December, 1927.

1. Chemotherapy of bubonic plague—Caius and Naidu.
2. A method for standardising Haffkine's plague prophylactic—Naidu and Shamsher Jung.
3. A discussion on the treatment of bubonic plague in India with special reference to d'Herelle's anti-pestiphage—Avari and Naidu.

Enquiry on maternal morbidity and mortality in childbirth.

1. *Anæmia of pregnancy.*—One hundred and seven cases were followed up, of which 76 were seen in Bombay Hospitals, 14 in Calcutta Hospitals and 17 in Madras Hospitals. No marked difference was found in the clinical or blood conditions in the different towns.

(1) *Blood examination.*—Cultures were made in 16 cases, all were sterile. Van den Bergh's test was done in 32 cases. Of these (according to the indirect method) 2 were negative. The remainder gave an average indirect reaction of 0·69 units. Nine of these gave a reaction above unity, the highest being 2·0 units. Three gave immediate results, but 2 of these were nonpregnant anæmias, while the third suffered from ankylostome. The blood calcium was estimated in 17 cases of which 4 were probably secondary anæmias. In the remaining 13 the average amount was 9·3 mgrs. Only 3 were below 9 mgrs. (7·5, 8·7 and 8·9 mgrs. respectively).

Khan's test for syphilis was done in 42 cases of which 11 were positive and 31 negative. The blood count and blood picture were the same as described in former reports.

(2) *Urine examination.*—Sixty-nine cases were examined for albumen; thirty-eight were positive and 31 negative. In 63 cases the urine was cultured with the following results:—

Streptococci present in	27	cases or	42·0 per cent.
Other organisms	21	,,	33·0 per cent.
Sterile	16	,,	25·0 per cent.

In last year's report the percentage of streptococci found was 6·5 only. The difference is due to different methods of culture. This year the urine has been either cultured whole or in glucose broth. The number of cases in which streptococci were found in the urine in Madras were unusually high, that is, in 9 out of 14 examined.

Some biochemical tests were kindly done by Major Sokhey. In 3 cases the urea concentration test was done and showed a fairly normal kidney. In 2 cases the levulose tolerance test was done and indicated some abnormality of the liver.

The number of cases of normal pregnancy examined antepartum as controls was brought up to 59.

The percentage sterile was 91·6 per cent.

(3) *Fæces examination.*—(*a*) For ova :—

Hookworm	8
Roundworm	5
Whipworm	2
Negative	36
	51

(*b*) For *B. welchi.*—Forty-five cases of anæmia and 31 normal cases had their stools examined, and *B. welchi* was separated in pure culture. Observations were made on their method of separation, their cultural reactions, the preparation of toxin and animal reactions. It was found that anæmia could be produced in guineapigs in some cases by the injection of these toxins also that premature and still-births were often produced. These experiments are being continued.

(*c*) For streptococci.—Two *post mortem* examinations were made during the year.

II. *Still-birth enquiry.*—Two hundred and fifty cases of still-birth were examined in Bombay Hospitals. Of 146 cases tested for syphilis by Khan's test 68 were positive and 78 were negative, that is 46 per cent. were positive as compared with 15 per cent positive in a series of 100 cases of normal pregnancy. It cannot of course be definitely stated that syphilis was the cause of the child's death, as the examination was of the maternal blood only, not of the fœtus.

The causes of fœtal death were estimated as follows :—

Complications of labour	23 per cent.
Maternal syphilis	17 per cent.
Maternal toxæmia (including antepartum hæmorrhage) ...	5 per cent.
Other maternal and fœtal conditions...	6 per cent.
Unknown causes	46 per cent.

Many of the 46 per cent. put down to unknown causes were severe or slight cases of anæmia, or gave a history of past fever which might or might not have been malarial. On comparing these results with similar results published in England, it appears that in India complications of labour are a smaller cause of still-birth, and maternal disease is a larger cause.

III. *Maternity conditions of women industrial workers.*—Observations have been made on 229 cases of childbirth in this class but the report is being held back until a larger number have been collected.

IV. *Age of primiparæ in India.*—847 primiparæ in Bombay averaged 18·7 years. 2,312 primiparæ in Madras averaged 19·4 years. 90 primiparæ in Ahmednagar averaged 18·2 years. 1·2 per cent. of the Madras mothers, 2·1 per cent. of the Bombay mothers, and 7·7 per cent. of the Ahmednagar mothers were below 16 years of age.

V. *Infant birth weight in India.*—In 1609 full time healthy infants the average birth weight was 6·2 lbs.

VI. *The following papers were read or published*:—

(1) Anæmia of Pregnancy; by Dr. M. I. Balfour, Indian Medical Gazette, September 1927.

(2) Maternal mortality in Childbirth in India; by Dr. M. I. Balfour, Indian Medical Gazette, November 1927.

(3) Some diseases of pregnancy in India; by Dr. M. I. Balfour, read at the Obstetrical section of the F.E.A.T.M. Conference, December 1927.

(4) A preliminary note on bacteriological examination of some discharges and excreta from cases of anæmia of pregnancy, normal pregnancy and non-pregnant cases of anæmia; by Dr. Alexandra Emanuelov and Dr. Maneck Mehta, read at the F.E.A.T.M. Conference, Obstetrical section, December 1927.

Schistosomiasis

A disease, analogous to human bilharziasis (*Schist. mansoni* and *Schist. japonicum* which is not endemic in India), is prevalent in certain localities among herbivorous animals mostly goats, sheep, and bullocks. It is caused by a parasitic worm, *Schist. spindalis* which has the same mode of existence and propagation as its prototypes *S. mansoni* and *S. japonicum* and the disease runs a like course in the infected animals.

Dr. N. H. Fairley and S. A. S. Jasudasan have been carrying on an enquiry on this disease since 1920 in its various aspects such as etiology, morbid histology, serology and therapy, to elucidate some problems of common interest in these diseases and have contributed valuable information to its literature. In Dr. Fairley's absence further work has been carried out by S. A. S. Jasudasan and Col. Mackie according to a scheme framed by Dr. Fairley for the investigating of the following features.

(1) The peculiarities in the transmissibility of the disease through the upper alimentary tract, especially the mouth and stomach, as contrasted with this means as a portal of infection in human disease. The pathological changes produced at the areas of penetration, cutaneous and mucosal, by cercariæ.

(2) The antigenic value of cercarial antigen as compared with that of adult schistosome worms, the thermostabile character of the routine specific antigen, a reagent used in Dr. Fairley's complement fixation test in Bilharziasis and the antigenic factor in its constituents.

(3) If the natural immunity gained by the blood of monkeys (*Macacus sinicus*) against *S. spindalis* by virtue of which the latter are killed, in vivo, after the first infection, has any effect on the cercariæ in the subsequent infections. The action, in vitro, of such immune sera on cercariæ.

(4) The therapeutic effect of antimony tartrate in producing a complete cure to the extent of converting a positive phase of the complement fixation reaction in Bilharziasis to a negative one which is the criterion of a radical cure.

(5) Effect of such antimony tartrate alone, or antimony and serum, on cercariæ has been tested in vitro to find out if the antimony directly, or in combination with some constituents of serum, exerts a lethal action on the schistosome.

(6) The therapeutic value of Neostam or stibamine glucoside (B. W. & Co.) in Bilharziasis.

In addition the complement fixation reaction on the sera of a series of rabbits artificially immunised against various types of *Monilia ashfordi*, the supposed agent in sprue and on the sera of patients who have suffered from sprue was also done.

Details of the enquiry will appear in the Indian Journal of Medical Research in due time.

The following papers on this subject have already appeared in the press:—

1. A new Serological reaction for *S. spindalis* and its practical value; Trans. Indian Science Congress, 1923.
2. Complement fixation with Bilharzia, Part I—The Lipoidal nature of cercarial antigen as used in the Complement Fixation test for mammalian Bilharziasis; Journal of Pathology and Bacteriology XXVIII, 591.
3. Studies in the Chemotherapy and Immunity reactions of Schistosomiasis (*S. spindalis* and *S. hæmatobium*); Transactions of the Royal Society of Tropical Medicine and Hygiene, 1926, Volume XX, No. 4.
4. The early spontaneous cure of Bilharziasis (*S. spindalis*) in Monkeys (*Macacus sinicus*) and its bearing on species immunity; Indian Journal of Medical Research, 1926-1927, Volume XIV.
5. The experimental Transmission of *S. spindalis* to the Indian water buffalo; Indian Journal of Medical Research, 1926-1927, Volume XIV.

APPENDIX

ANTIRABIC TREATMENT

TABLE I

Statistics for persons treated at Parel, 1922-1927

Year	Total treated	Failures		Deaths		Indians			Europeans			Cases extracted Advice cases and incomplete treatment
		Total No.	Per cent.	Total No.	Per cent.	Total No.	Deaths	Per cent.	Treated	Deaths	Per cent.	
1922	429	4	0·93	4	0·93	365	4	1·09	64	...	...	212
1923	845	9	1·06	10	1·18	730	10	1·37	115	...	...	523
1924	587	7	1·19	10	1·70	525	9	1·71	62	1	1·61	513
1925	625	5	0·8	6	0·96	583	6	1·02	42	...	...	355
1926	648	4	0·6	5	0·76	588	5	0·87	60	...	...	611
1927	729	4	0·55	5	0·68	678	5	0·73	51	...	...	644

TABLE II

Classification of patients by probability of rabies in the biting animals

Class	Sub-class I Bitten on head or face	Sub-class II Bitten through bare skin anywhere except on the head or face	Sub-class III Bitten through clothing	Sub-class IV "Licks" on any part of the body	Total	Deaths	Mortality per cent.
Class A—Bitten or licked by animals proved rabid ...	...	43 (1)	6	33	82	1	1·22
Class B—Bitten or licked by animals certified rabid ...	...	12	1	...	13	...	...
Class C—Bitten or licked by animals suspected rabid ...	25	468 (4)	133	8	634	4	0·63
Total ...	25	523 (5)	140	41	729	5	0·67
Europeans							
Class A—Bitten or licked by animals proved rabid ...	...	11	2	17	30	...	...
Class B—Bitten or licked by animals certified rabid ...	...	1	...	...	1	...	...
Class C—Bitten or licked by animals suspected rabid ...	2	11	6	1	20	...	...
Total ...	2	23	8	18	51	...	...
Indians							
Class A—Bitten or licked by animals proved rabid ...	...	32 (1)	4	16	52	1	1·92
Class B—Bitten or licked by animals certified rabid ..	...	11	1	...	12	...	...
Class C—Bitten or licked by animals suspected rabid ..	23	457 (4)	127	7	614	4	0·65
Total ...	23	500 (5)	132	23	678	5	0·73

TABLE III

Cases developing hydrophobia within 15 days of completion of treatment

Case No.	Sex	Age	Animal	Number and position of the bite.	Whether on bare skin or through clothing	Whether cauterized or not	Date of commencing treatment	Number of days late	Duration of illness	Interval between bite and death	Remarks
1264	M	5	Dog ...	1; arm.	Bare...	Uncauterized.	24th November 1927.	0 days.	3 days.	21 days.	

TABLE IV

Cases developing hydrophobia after 15 days of completion of treatment "Failures"

Case No.	Sex	Age	Animal	Number and position of the bite	Whether on bare skin or through clothing.	Whether cauterized or not	Date of commencing treatment	Number of days late	Duration of illness	Interval between bite and death	Remarks
389	M	14	Dog ...	4; arm.	Bare...	Cauterized ...	1st April 1927.	0 days.	2 days.	88 days.	
514	M	20	,, ...	4; leg .	,, ...	Uncauterized.	30th April 1927.	4 days.	,, ...	43 days.	
610	M	8	,, ...	4; arm.	,, ...	Cauterized ...	20th May 1927.	1 day .	,, ...	30 days.	
950	M	4	Hyena.	5; leg .	,, ...	Do. ...	11th Aug. 1927.	28 days.	,, ...	90 days.	

TABLE V

Showing the animals which bit, scratched or licked the patients

Species of animal	Number of persons bitten or scratched or licked	Deaths
Dog	690	4
Jackal	15	.....
Human being	1	.....
Horse	2	.....
Cat	12	.....
Monkey	7	.....
Hyena	2	1
Total ...	729	5

TABLE VI

Showing the number of persons who came for treatment each month during the year 1927

Month	Discontinued treatment	Europeans		Indians	
		Treated cases	Advice cases	Treated cases	Advice cases
January	22	11	4	61	47
February	10	9	...	58	47
March	9	6	3	52	47
April	13	3	4	55	52
May	20	3	2	84	51
June	15	6	6	75	36
July	11	3	2	42	30
August	12	2	1	42	36
September	8	...	7	60	29
October	9	5	3	45	35
November	7	3	2	54	27
December	11	...	3	50	31
Total ...	147	51	37	678	468

TABLE VII

Total number of patients treated at Parel according to provinces from which they came

Province	Europeans	Indians	Total
Bombay City	50	613	663
Bombay Presidency	.....	53	53
Central Provinces	.....	4	4
Rajputana	1	.....	1
Goa	.....	5	5
United Provinces	.....	1	1
Indian States	.....	2	2
Total ...	51	678	729

Digitized with financial assistance from the Government of Maharashtra on 11 July, 2017

TABLE VIII

Statement of cases treated at different centres during 1927

Name of the place	Number of persons for whom vaccine was issued	Number of persons who received the full course of treatment	Number of persons not fully treated	Number of reports received from persons alive and well at least for three months	Died during treatment	Died less than fifteen days after completing treatment	Died more than fifteen days after completing treatment	Number of subsequent reports received
Ahmedabad ...	788	734	54	...	2	...	...	732
Ahmednagar ...	139	139	...	...	...	...	...	139
Ajmere ..	160	154	6	133	...	1	2	18
Akola ...	164	150	14	64	...	...	...	86
Baroda ...	129	107	21	44	1	...	...	62
Belgaum ..	152	151	1	90	...	...	...	61
Bombay (Byculla and Colaba) ...	336	325	11	325	...	...	...	...
Cambay ...	8	7	1	7	...	...	...	...
Chhota Udaipur	44	39	5	38	...	1	...	...
Dehra Dun ...	28	28	...	27	...	...	1	...
Hoshangabad ...	184	176	8	...	...	...	...	176
Hyderabad ..	44	36	8	7	...	...	...	29
Hubli ...	28	25	3	23	...	...	2	...
Indore ...	197	177	20	...	1	1	...	175
Jalgaon ...	79	69	10	68	...	1	...	...
Jhansi ...	51	49	2	25	...	...	...	24
Jubbulpore ...	284	280	20	26	...	1	...	253
Karachi ...	174	182	28	180	...	...	...	2
Karwar ...	13	13	...	11	...	...	2	...
Kolhapur ...	8	4	4	4	...	...	...	...
Meerut ...	14	9	5	9	...	...	...	...
Mhow ...	5	5	...	5	...	...	...	...
Nagpur ...	337	318	19	15	1	1	...	301
Nasik ...	43	29	14	20	...	...	...	9
Nasirabad ...	25	25	...	25	...	...	...	...
Nova Goa ...	66	66	...	24	...	...	1	41
Poona ...	392	271	121	2	...	...	...	269
Quetta ..	77	67	10	25	...	...	...	42
Raipur ...	76	73	3	..	...	...	...	73
Rajpipla ...	3	3	...	...	...	...	...	3
Rajkote ...	356	336	20	211	...	5	1	119
Ratnagiri ...	6	5	1	5	...	...	...	...
Sholapur ...	21	13	8	9	...	...	...	4
Surat ...	168	95	73	44	...	...	...	51
Total ...	4,599	4,160	490	1,466	5	11	9	2,669

BOMBAY : PRINTED AT THE GOVERNMENT CENTRAL PRESS.